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Mapping quantitative trait loci for seedling vigour and development in sunflower (*Helianthus annuus* L.) using recombinant inbred line population

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Abstract

The ability of seeds to germinate and establish seedlings in a predictable manner under a range of conditions has a direct contribution to the economic success of commercial crops, and should therefore be considered in plant breeding programs. Quantitative trait loci (QTLs) implicated in seedling vigour and development of sunflower were investigated using a population of recombinant inbred lines (RILs) developed through single-seed descent from the cross 'PAC2 × RHA266'. The experiments were conducted in completely randomized design with three replications. Different traits associated with germination, seedling vigour, early growth and development were studied. Analysis of variance for all studied traits revealed significant differences (P<0.01) among RILs and their parents. A large genetic variation and transgressive segregation was observed for all the studied traits. QTL-mapping was performed using a high-density simple sequence repeat/ amplified fragment length polymorphism (SSR/AFLP) linkage map and several QTLs associated with the studied traits were identified. The percentage of phenotypic variation explained by individual QTLs ranged from 5.4% to 35.7%. Based on overlapping support intervals, the co-location of QTLs for all ten traits was determined. QTLs controlling most of the traits were overlapped on different linkage groups, which was in accordance with the phenotypic correlation results among the traits. Although the detected regions need to be fine-mapped to dissect the genes underlying QTLs, the information obtained could help sunflower breeders in marker-assisted breeding programs for developmental traits.

Keywords: genetic variation, germination, seedling development, transgressive segregation, QTLs co-location.
Abbreviations: AFLP: amplified fragment length polymorphism, CIM: composite interval mapping, DSE: days to seedling emergence, DRW: dry root weight, DSW: dry shoot weight, FRW: fresh root weight, FSW: fresh shoot weight, GLM: general linear model, HD: hypocotyle diameter, QTL: quantitative trait loci, RILs: recombinant inbred lines, RootL: root length, ShootL: shoot length, SSD: single seed descent, SSR: simple sequence repeat, Sger: speed of germination.

Introduction

Sunflower (*Helianthus annuus* L.) is grown mostly as a source of vegetable oil and proteins in many countries throughout the world (Leon et al., 1995; Hu et al., 2010). Seed vigour has been described as the sum total of those properties of the seed that determine the potential level of its activity and performance during germination and seedling emergence (Perry, 1978). Seedling height and dry weight have been identified as good indicators of seed vigour (Regan et al., 1992). Seed vigour is complex adaptive trait of higher plants that are influenced by both genetic and environmental components (Whittington, 1973; Hodgkin and Hegarty, 1978). Although the environmental influences on seed vigour have been extensively studied (Sinniah et al., 1998), the genetic components are less understood and documented. If the genetic loci of seed vigour and seedling development were identified, plant breeders would be able to select specifically for alleles contributing to improved seed vigour and seedling development. Seedling vigour is a trait that expresses itself as an ability of seedlings to rapidly elongate after germination and emerge for escaping and surviving submergence stress (Vu et al., 2010). Seedling vigour is one of the major determinants for successful crop establishment (Zhang et al., 2005). The availability of locus-specific molecular markers for seed vigour would be of great benefit, providing the potential for more rapid screening of beneficial combinations of alleles in breeding programs. Molecular markers have been successfully used for tagging genes contributing to polygenic traits through QTL-mapping. The technique integrates molecular marker data with data obtained on quantitative traits to give information on the effects and locations of the loci controlling quantitative traits (Tanksley, 1993; Bohuon et al., 1998; Kearsey, 1998). Several molecular genetic linkage maps has been constructed and used for dissecting complex traits in sunflower,

specifically using recombinant inbred lines (RILs) population (Berry et al., 1995; Jan et al., 1998; Gentzbittel et al., 1999; Poormohammad Kiani et al., 2007). RILs populations are very useful because each genotype can be tested repeatedly and by applying different test systems. The latter allows studying the pleiotropic effects of loci, which are suggested to be due to the co-location of QTLs for different traits. Identification of chromosomal regions controlling seed dormancy, seed shape, seed germination, seed vigour, seedling vigour and development has been reported in different species, e.g. rice (Lin et al., 1998; Clerkx et al., 2004; Bettey et al., 2000; Redona et al., 1996; Lu et al., 2007; Ray et al., 1996, Yadav et al., 1997; Price et al., 2000 Hossain et al., 2010). To our knowledge, little information about identification of chromosomal regions effecting seed germination and seedling vigour and development of sunflower are available. In a study, Rachid Al-Chaarani et al. (2005) reported a number of QTLs for traits corresponding to seedling vigour and development such as: shoot length, root length, dry root weight, dry shoot weight, fresh root weight, fresh shoot weight and percentage of normal seedlings in sunflower. The objectives of present study were to determine the genetic variability and QTLs involved in seedling vigour and development in sunflower using a high density simple sequence repeat/ amplified fragment length polymorphism (SSR/AFLP) developed genetic linkage map on recombinant inbred line population.

Results

Phenotypic analysis

Analysis of variance for all the studied traits revealed significant differences (P<0.01) among genotypes (Table 1). The differences between parental lines were not significant for the studied traits (Table 2). The difference between RILs ($\overline{X}_{RIL}$), and the mean of their parents ($\overline{X}_{P}$), was also not significant (Table 2). The difference between the mean of parents ($\overline{X}_{P}$) and the mean of 10% superior RILs ($_{10\%SRIL}$) were significant for all the studied traits except for dry shoot weight (DRW) (Table 2). Difference between the best parent ($\overline{X}_{BP}$) and the best RIL ($\overline{X}_{BRIL}$), was significant for all the studied traits except for speed of germination (Sger). Broad sense heritabilities were low to moderate, comprised between 31 to 49%. Frequency distribution of RILs and their parents for most of the studied traits showed continuous patterns with approximately normal distribution, suggesting that germination and developmental traits are controlled by a polygenic system (Figure 1). Some RILs showed less value for the studied traits than their parents, whereas some others showed higher value than them (Figure 1).

Correlation study

The correlation coefficients among the traits were given in Table 3. We found significantly positive correlations among some studied traits. Days to seedling emergence (DSE) was significantly correlated with days to growth stage V4. The correlations between root length (RootL) and shoot length (ShootL), fresh root weight (FRW) and fresh shoot weight (FSW), between ShootL and fresh shoot weight (FSW), dry root weight (DRW) and dry shoot weight (DRW) were highly significant (P<0.01). Similarly, the correlation coefficients between fresh root weight (FRW) and fresh shoot weight, (FSW) dry shoot weight (DSW) as well as between fresh shoot weight (FSW) and dry root weight (DRW), dry shoot weight (DRW) were positively significant.

Quantitative trait loci analysis

The map position and characteristics of QTLs associated with the studied traits are summarized in Table 4. The QTLs were designated as following: speed of germination 'Sger', days to seedling emergence 'DSE', hypocotyle diameter 'HD', shoot length 'ShootL', root length 'RootL', dry root weight 'DRW', dry shoot weight 'DSW', fresh root weight 'FRW', fresh shoot weight 'FSW' and V4 developmental stage 'V4', followed by the corresponding number of linkage group (LG) and the corresponding number of QTLs in the group. For an easier overview of overlapping QTLs between traits, an image of all QTL regions is presented in Supplementary data 1. Fifty eight putative QTLs associated with ten studied traits were detected using studied RILs population through composite interval mapping technique. The sign of the additive effects show that both parental lines contributed to positive alleles for germination and developmental traits. Overlapping QTLs were found for different traits on several linkage groups (Table 5 and Supplementary data 1). Two QTLs were detected for speed of germination (Sger). These QTLs were located on LGs 6 and 10 and percentage of explained phenotypic variation (R^2) was 8.2 and 11.7%, respectively. Both parental lines contributed almost equally in trait expression. Seven QTLs associated with the days to seedling emergence (DSE) were detected on LGs 3, 5, 8, 10 and 16 with R^2 from 7.3 to 10.6%. PAC2 contributed at five QTLs. QTL analysis revealed 7 loci on LGs 3, 5, 7, 10 and 16 for hypocotyle diameter (HD) with R^2 varying from 7.4 to 16.5%. The positive alleles for five QTLs come from PAC2 and 3 out of 7 QTLs were overlapped with the QTLs of other traits. Five QTL were identified for V4, on LGs 3, 5, 7 and 14 explaining from 5.4 to 12.2% of phenotypic variation. The positive alleles for the most of QTLs come from PAC2. Four QTLs for ShootL were found on LGs 8, 12, 13, and 16, explaining 7.1 to 18.1% of the phenotypic variation. Three out of 4 QTLs were identified being specific to ShootL. Both parental lines contributed to trait expression. For RootL, three QTLs on LGs 3, 8 and 16, explaining 11.9 to 14.4% of the phenotypic variations were detected and 2 QTLs overlapped with dry root weight (DRW) and HD QTLs on LGs8 and 16. A total of 8 QTLs were found for fresh shoot weight (FSW) on LGs 1, 3, 6, 7, 10, 12 and 17. These QTLs explained 9.6 to 21.0% of the phenotypic variation. Four out of 8 QTLs were revealed being specific to FSW. PAC2 and RHA266 contributed equally to QTLs involving in FSW phenotype. Ten QTLs were detected for dry shoot weight (DSW), responsible for 12.8 to 18.0% of the phenotypic variation. PAC2 contributed to most of the positive alleles. Three out of ten QTLs located on LGs 5, 7 and 9 were specific to DSW and the rest were co-localized with DSE, fresh root weight (FRW) and FSW QTLs on LGs 3, with FRW, FSW, ShootL and HD QTLs on LGs 10, with FSW QTLs on LGs 12, with V4 QTLs on LGs 14, with FSW, HD QTLs on LGs 16 and with FRW ones on LGs 17. QTL analysis revealed 5 genomic regions controlling FRW phenotype variation, on LGs 3, 10, 12, 15 and 17. Variation explained by individual QTLs ranged from 9.8 to 11.3%. Two QTLs were specific to FRW and most of the positive alleles come from RHA266. For DRW, 7 QTLs were detected on LGs 1, 2, 7, 8, 9, 12 and 15 with R^2 varying from 7.9 to 30.0%. Five out of 7 QTLs were unique. Four positive alleles come from RHA266 and 3 come from PAC2. Overall, individual QTLs had minor effect on

Table 1. Mean squares for germination and seedling vigour, growth and developmental traits in sunflower recombinant inbred lines (RILs) and their two parents studied in controlled conditions.

Source	V4		DSE		FRW	FSW	DRW	DSW	RootL	ShootL	HD		Sger	
	df	MS	df	MS	df	MS	MS	MS	MS	df	MS	MS	df	MS	df	MS
REP	5	232.26**	5	16.53**	2	0.003	0.003*	0.00002	0.0000008	11	9.83*	8.10^{ns}	5	0.0003^{ns}	2	0.001^{ns}
RIL	115	35.31**	115	8.08**	77	0.01**	0.01**	0.00006**	0.00004*	81	73.24**	20.99**	115	0.003**	89	0.065**
Error	562	1.58	588	0.21	160	0.003	0.0008	0.00003	0.00001	896	4.91	2.61	581	0.0004	182	0.009
CV		4.34		5.52		48.92	16.02	92.29	40.55		32.41	24.93		8.94		10.92

d.f.: degrees of freedom; MS: mean of square; CV: coefficients of variation. The traits are: Speed of germination (Sger), days to seedling emergence (DSE; day), hypocotyle diameter (HD; cm), shoot length (ShootL; cm), root length (RootL; cm), dry root weight (DRW; g), dry shoot weight (DSW; g), fresh root weight (FRW; g), fresh shoot weight (FSW; g), and V4 growth stage (V4; day). *: Significant at 0.05 level, **: Significant at 0.01 probability level; ns: non significant.

Table 2. Genetic gain and heritability for germination and seedling vigour, growth and developmental traits in sunflower recombinant inbred lines (RILs).

Parameter	V4	DSE	FRW	FSW	DRW	DSW	RootL	Shoot L	HD	Sger
PAC-2 (P1)	26.17	9.00	0.04	0.11	0.0021	0.0053	5.32	4.73	0.21	0.87
RHA266(P2)	26.83	8.00	0.06	0.12	0.0024	0.0061	5.92	5.70	0.24	0.27
P1-P2	-0.66	1.00	-0.02	-0.01	-0.0003	-0.0008	-0.6	-0.97	-0.03	0.60
$\overline{X}_{P}$=(P1+P2)/2	26.5	8.5	0.05	0.115	0.0022	0.0057	5.62	5.22	0.225	0.57
$\overline{X}_{RIL}$	29.02	8.39	0.12	0.18	0.01	0.01	6.86	6.53	0.23	0.87
$\overline{X}_{RIL}$- $\overline{X}_{P}$	2.52	-0.11	0.07	0.065	-0.012	0.0043	1.24	1.32	0.005	0.3
$\overline{X}_{BRIL}$	22.83	6.67	0.38	0.344	0.04	0.0212	12.86	9.79	0.30	1.00
GGB= $\overline{X}_{BRIL}$- $\overline{X}_{BP}$	-3.34*	-1.33*	0.31*	0.224*	0.038*	0.015*	6.94*	4.09*	0.06*	0.13^{ns}
$\overline{X}_{10\%SRIL}$	24.41	6.99	0.24	0.29	0.01	0.02	11.84	9.01	0.27	0.96
GG10%= $\overline{X}_{10\%SRIL}$- $\overline{X}_{P}$	-2.09*	-1.51*	0.19*	0.18*	0.008^{ns}	0.01*	6.22*	3.79*	0.05*	0.39*
h_n^2	0.48	0.49	0.38	0.46	0.31	0.39	0.47	0.45	0.44	0.44
LSD0.05	1.4224	0.5186	0.0877	0.0453	0.0088	0.0051	1.7731	1.2927	0.0226	0.1518

Speed of germination (Sger), days to seedling emergence (DSE; day), hypocotyle diameter (HD; cm), shoot length (ShootL; cm), root length (RootL; cm), dry root weight (DRW; g), dry shoot weight (DSW; g), fresh root weight (FRW; g), fresh shoot weight (FSW; g), and V4 growth stage (V4; day). $\overline{X}_{P}$: Mean of parents. $\overline{X}_{RILs}$: Mean of all recombinant inbred lines (RILs). 10%SRILs: Mean of the 10% Selected RILs for each measured characters. GG10%: Genetic gain when the mean of 10% selected RILs is compared with the mean of parents. $LSD_{0.05}$: Least significant differences calculated using $t_{0.05}$ and error mean square of each experiment. * and ns: significant at 0.05 level and non significant. h^2: broad -sense heritability.

phenotypic variation and both parental lines contributed in QTL expression of traits.

Discussion

Phenotypic variation among genotypes

A large genetic variation was observed among RILs population for the studied traits (Table 1). These findings are in agreement with previous reports on quantitative genetic analysis of seedling vigour and development in sunflower (Rachid Al-Chaarani et al., 2005). Lu et al. (2007) identified a significant difference for seedling early vigour traits in rice. In wild oat population, 50% of phenotypic variation for germination was due to the influence of genetic factors (Whittington, 1973). The frequency distribution of RILs and their parents for germination and seedling growth and development showed a continuous pattern, suggesting that these traits are controlled by a polygenic system. Continuous variation was also observed for seedling early vigour traits in rice (Lu et al., 2007). The difference between mean of RILs ($\overline{X}_{RIL}$), and the mean of their parents ($\overline{X}_{P}$), is not significant for all studied traits (Table 2), indicating that the RILs used in this study are representative of the entire possible recombinant lines from the cross 'PAC2×RHA266'. Genetic gain (positive transgressive segregation) presented as the differences between the mean of best parent ($\overline{X}_{BP}$) and the mean of best RIL ($\overline{X}_{BRIL}$) or between the mean of 10% selected RILs ($\overline{X}_{10\%SRIL}$) and the mean of the parents ($\overline{X}_{P}$), was significant for the most of the traits (Table 2). This phenomenon might be due to the polygenic nature of the traits and the accumulation of favorable alleles from both parental lines in RILs. The positive and negative signs of additive effect at the different QTLs confirm the contribution of both parental lines and the transgressive segregation observed at the phenotypic level (Table 2). Transgressive phenotypes have also been reported for some growth and developmental traits in sunflower by Rachid Al-Chaarani et al. (2005) and Poormohammad Kiani et al. (2009). Betty et al. (2000) reported transgressive segregation for seed vigour and pre-emergence seedling growth traits in *Brassica oleracea.* Transgressive segregation for seedling early vigour traits has been reported in *Oryza sativa* L. (Lu et al., 2007). Broad-sense heritability for germination and developmental traits varied between 0.31 to 0.48 indicating that selecting for these traits in segregating populations through conventional phenotypic selection should be complex and therefore marker-assisted selection (MAS) could be an effective tool in breeding programs.

Traits correlations

Positive and significant correlations were observed among studied traits (Table 3). A strong correlation between plant growth and developmental traits has been reported in Rachid Al-Chaarani et al. (2005) research work on sunflower. Cui et al. (2002) observed significant correlations among seedling vigour traits in rice. These researchers identified several common chromosomal regions controlling seedling growth characteristics and seed sizes. In the present study, the correlation coefficient between some developmental traits, e.g. plant shoot length and fresh and dry shoot weight was significant. These traits shared some QTLs with additive effects in the same directions, thus leading to a conclusion that plant shoot length partially shares a genetic basis with fresh and dry shoot weight. Conventional quantitative genetic suggest that trait correlations may be attributable to either pleiotropic effects of single genes or to tight linkage of several genes that individually influence specific traits. Thus, it is expected that correlated traits have QTLs mapping to the same genomic regions (Veldboom et al., 1994). The same trend was observed in our study. There was no correlation between germination parameter and most growth and developmental traits. Collectively the results presented indicate that germination performance and the seedling growth and developmental stages in sunflower are under separate genetic control.

QTL mapping of germination and seedling early vigour traits

A total of fifty eight QTLs were found to be associated with seed germination, seedling vigour, growth and developmental traits (Table 4). The percentage of phenotypic variance (R^2) explained by identified QTLs ranged from 5.4% to 35.7% and both parental lines (PAC2 and RHA266) contributed to positive alleles for traits (Table 4). The contribution of positive alleles from both parental lines resulted in RILs with higher values for the traits than those of their parents. For shoot length (ShootL) QTLs with low individual effects were identified. QTLs detected for dry root weight (DRW), dry shoot weight (DSW), fresh root weight (FRW) and fresh shoot weight (FSW) each also explained low individual phenotypic variation (Table 4). Rachid Al-Chaarani et al. (2005) reported a number of QTLs for shoot length, root length, dry root weight, dry shoot weight, fresh root weight, fresh shoot weight and percentage of normal seedlings that some were on the same linkage groups and sometimes close together. A similar work was realized in rice and a total of 31 QTLs were detected for germination rate, total dry weight, shoot dry weight, root dry weight and maximum root length, accounting for rather high total phenotypic variation (Cui et al., 2002). Also significant QTLs were detected for shoot weight, root growth rate and root length in *B. oleracea* (Betty et al., 2000). Paterson and Sorrells (1990) found multiple dominant genes responsible for late germination. Based on overlapping support intervals, the co-location of QTLs for all ten traits were determined. One of important example, interval E40M50_2-HA2920 on LG3 was significantly associated with various traits (Supplementary data 1). In this interval, the QTLs controlling DSE, DSW, FRW and FSW were overlapped (Supplementary data 1). Similarly, several overlapping QTLs were also observed for the studied traits (Table5) confirming the phenotypic correlation results among these traits. These overlapping QTLs indicate the existence of a partly common genetic base for related traits. The common genetic basis might be the pleiotropic effects of a single and/or limited number of QTL or tightly linked loci controlling these traits. Pleiotropic effects or close linkage of gene(s) are the main causes for correlations among traits (Aastveit and Aastveit, 1993). Identification of QTLs influencing several traits could increase the efficiency of MAS and enhance genetic progress (Upadyayula et al., 2006). The correlation among different traits as well as their co-localized QTLs observed in our study is relevant to effort for manipulating multiple traits simultaneously. As we identified genetic markers linked to seedling vigour traits, indirect selection can be targeted at the presence or absence of markers of interest in breeding programs. Molecular markers linked to the QTLs offer a rapid and efficient

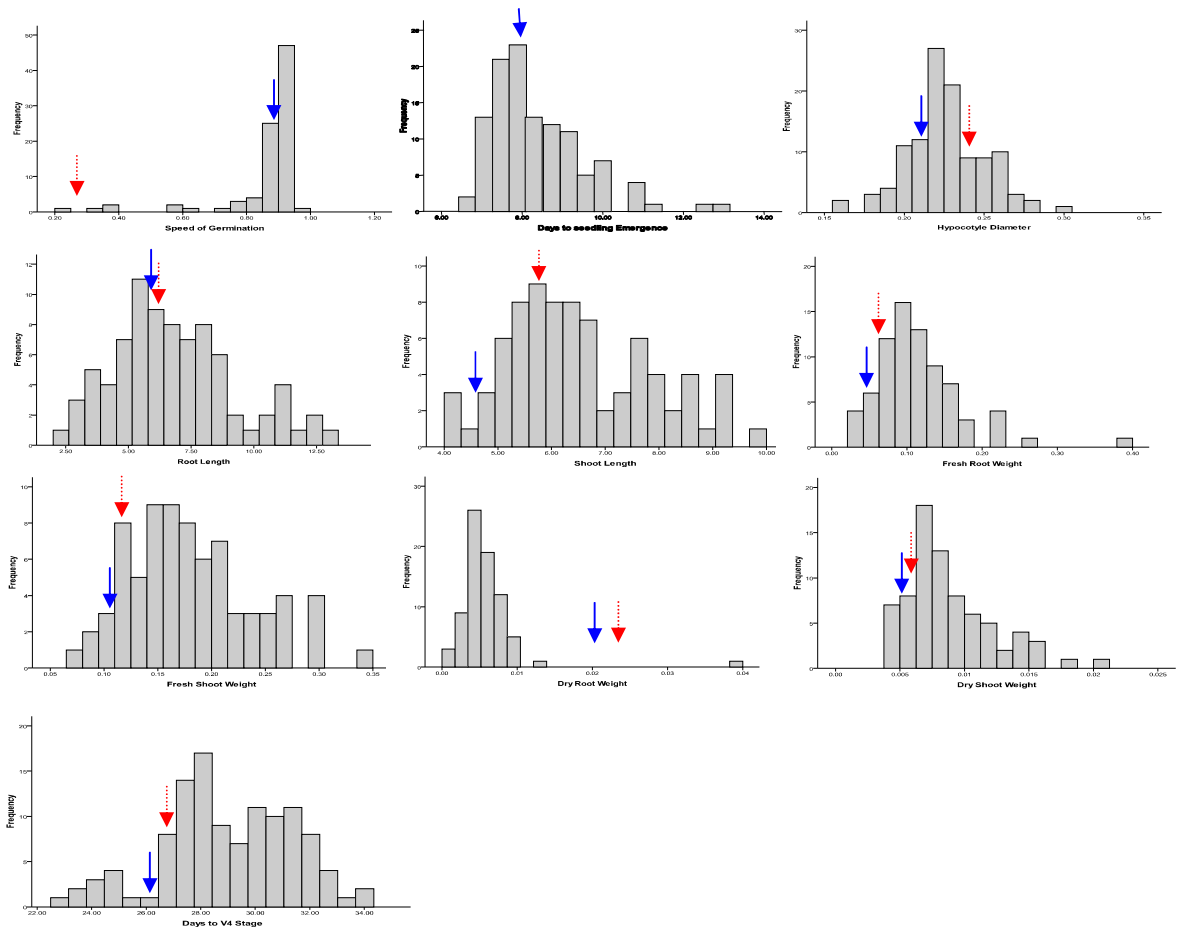

Fig 1. Frequency distribution of sunflower recombinant inbred lines (RILs) and their parents for germination and seedling vigour growth and developmental traits. *Arrows* show phenotypic values of parental lines (red arrows show: P1=PAC2 and blue arrows show: P2= RHA266) concerning to each characters.

Table 3. Simple correlation coefficients between different traits in the recombinant inbreed lines of sunflower (*Helianthus annuus* L.).

Traits	V4	Sger	DSE	RootL	ShootL	FRW	FSW	DRW
Sger	0.01							
DSE	0.36*	-0.15						
RootL	0.05	-0.03	0.00					
ShootL	0.09	0.14	0.00	0.44**				
FRW	0.00	0.07	-0.02	0.32**	0.21			
FSW	0.05	0.01	0.20	0.51**	0.58**	0.57**		
DRW	-0.01	-0.06	0.13	0.09	0.27*	0.18	0.27*	
DSW	-0.08	-0.14	0.11	0.23	0.26*	0.23*	0.54**	0.14

Speed of germination (Sger), days to seedling emergence (DSE; day), hypocotyle diameter (HD; cm), shoot length (ShootL; cm), root length (RootL; cm), dry root weight (DRW; g), dry shoot weight (DSW; g), fresh root weight (FRW; g), fresh shoot weight (FSW; g), and V4 stage (V4; day). * Significant at 0.05 probability level. ** Significant at 0.01 probability level.

selection gain for specific genotypes without extensive assessment of phenotype at all stages in the breeding programs (Cregan et al., 1999). However, the QTLs and related markers need to be validated prior to application in MAS. Most of the QTLs detected for the studied traits are co-localized whereas some of them were trait-specific (Supplementary data 1). We compared the position of QTLs identified in the present study with those identified by Rachid Al-Chaarani et al. (2005) for germination, growth and development traits in sunflower. One of QTLs for shoot length reported by Rachid Al-Chaarani et al. (2005) on linkage group 5 was confirmed in the present study on the corresponding linkage group 6 (Sger.6.1). Another QTLs on LG6 for root fresh weight and percentage of germinated seeds in Rachid Al-Chaarani et al. (2005) studies, were detected for DSW on linkage group 3 (DSW.3.1) in the present study. In the present study we identified the genomic regions controlling seed germination, seedling vigour, growth and developmental traits which overlapped with the QTLs previously reported for growth and yield-related traits (Poormohammad Kiani et al., 2009). As example, an overlapping QTL for days to seedling emergence with QTL

Table 4. QTLs detected for germination and seedling vigour, growth and developmental traits in sunflower recombinant inbred lines (RILs) and their two parents.

Trait	QTL	MARKR Name	[a] Position (cM)	LOD	Additive effects	R2	Trait	QTL	Position (cM)	LOD	Additive effects	R2
Sger	Sger.6.1	E32M49_24	53.16	7.7902	0.0765	0.117	FRW	*FRW.3.1*	E40M50_2	33.66	5.892	0.0216	0.0975
	Sger.10.1	ORS591	107.16	5.086	-0.0647	0.0815		*FRW.10.1*	SSL66_2	154.66	6.8436	-0.0216	0.1126
								FRW.12.1	HA3396	61.96	6.6818	0.0223	0.1116
DSE	*DSE.3.1*	E41M48_11	39.36	9.5544	-0.3975	0.1047		*FRW.15.1*	E35M61_5	17.81	6.083	-0.0209	0.1034
	DSE.5.1	SSL231	25.61	9.7654	-0.417	0.1142		*FRW.17.1*	ORS169	29.06	6.4703	0.0208	0.1054
	DSE.5.2	HA3627	79.81	13.2584	-0.4726	0.1462							
	DSE.8.1	ORS1043	5.81	6.4466	0.3517	0.0757	FSW	*FSW.1.1*	ORS803	0.01	10.8148	0.0307	0.2097
	DSE.8.2	ORS418_1	75.66	5.8605	-0.3384	0.0695		*FSW.3.1*	ORS718	31.61	5.4191	0.0213	0.1019
	DSE.10.1	E33M60_2	135.96	6.8062	-0.3353	0.073		*FSW.6.1*	HA4103	25.01	5.1927	-0.0206	0.0958
								FSW.7.1	ORS331_2	0.01	6.4752	-0.0241	0.1296
HD	*HD.3.1*	E32M49_1	40.71	7.0064	-0.0068	0.0736		*FSW.10.1*	HA1108	24.21	8.8648	0.0283	0.1782
	HD.5.1	ORS533	82.86	7.8179	0.008	0.103		*FSW.10.2*	SSL66_2	154.66	7.6758	-0.024	0.1293
	HD.7.1	HA3103	18.11	6.6537	-0.0069	0.0748		*FSW.12.1*	ORS1085_1	79.21	5.7294	-0.0211	0.099
	HD10.1	ORS437	99.61	6.9981	-0.007	0.0786		*FSW.17.1*	ORS169	29.06	6.5908	0.0218	0.1076
	HD.10.2	SSL66_2	158.66	13.7704	-0.0101	0.1648							
	HD16.1	HA3582	15.86	6.4086	-0.0068	0.0739	DRW	*DRW.1.1*	E40M50_18	18.11	5.1641	-0.0008	0.0833
	HD.16.2	E41M48_20	80.81	6.3804	-0.0064	0.0654		*DRW.2.1*	ORS653	50.36	5.6027	-0.001	0.1309
								DRW.7.1	E35M60_22	60.76	7.6835	0.0015	0.2955
Root L	*RootL.3.1*	E37M61_5	59.11	9.6801	0.8899	0.1366		*DRW.8.1*	E38M48_9	33.06	5.2032	0.0008	0.0786
	RootL.8.1	E38M48_9	35.06	8.1329	-0.9132	0.1437		*DRW.9.1*	ORS1009	11.61	6.4883	-0.001	0.1339
	RootL.16.1	ORS899	18.66	6.7062	0.8316	0.119		*DRW.12.1*	ORS671_2	21.11	12.2429	0.0013	0.2148
								DRW.15.1	ORS499	63.56	8.2749	0.0012	0.1865
Shoot L	*ShootL.8.1*	E37M61_2	22.31	12.458	-0.5438	0.1746							
	ShootL.12.1	ORS671_2	21.11	3.8465	0.3442	0.0708	DSW	*DSW.3.1*	E40M50_2	35.66	5.1124	0.0013	0.1403
	ShootL.13.1	E40M62_25	16.46	5.067	0.5505	0.1806		*DSW.5.1*	E37M61_10	14.31	5.8906	-0.0014	0.179
	ShootL.16.1	E41M48_43	169.51	8.8928	0.7737	0.3566		*DSW.7.1*	E35M60_10	48.96	8.2949	-0.0014	0.1591
								DSW.9.1	E38M48_5	98.46	8.4761	-0.0013	0.1363
								DSW.10.1	SSL66_2	154.66	8.05	-0.0013	0.1345
V4	*V4.3.1*	E41M48_11	39.36	5.3155	-0.5725	0.0581		*DSW.11.1*	ORS733	0.01	5.6398	0.0013	0.137
	V4.5.1	E35M60_1	25.56	9.8619	-0.7855	0.1084		*DSW.12.1*	SSU41_2	76.76	7.567	-0.0013	0.1367
	V4.5.1	ORS533	84.86	10.4458	-0.8361	0.1221		*DSW.14.1*	ORS391	75.36	5.0121	0.0013	0.1277
	V4.7.1	ORS1041	20.66	5.0709	0.5667	0.0542		*DSW.16.1*	E41M48_34	120.06	6.9706	-0.0012	0.1323
	V4.14.1	ORS1086_1	79.06	3.9076	-0.6026	0.055		*DSW.17.1*	ORS169	29.06	11.3163	0.0014	0.1781

[a] Expressed in cM, from north of the linkage group. Speed of germination (Sger), days to seedling emergence (DSE; day), hypocotyle diameter (HD; cm), shoot length (ShootL; cm), root length (RootL; cm), dry root weight (DRW; g), dry shoot weight (DSW; g), fresh root weight (FRW; g), fresh shoot weight (FSW; g), and V4 stage (V4; day). The QTLs were designated as the abbreviation of the trait followed by the corresponding number of linkage group (LG) and the corresponding number of QTLs on the group. The positive additive effect shows that PAC2 allele increases the trait and negative additive effect shows that RHA266 allele increases the trait.

Table 5. Overlapped QTLs controlling germination and seedling vigour, growth and developmental traits in sunflower identified on recombinant inbred lines population (RILs).

Linkage Group	Agronomical traits in the present study	Overlapped QTLs	Linkage Group	Agronomical traits in the present study	Overlapped QTLs
LG3	Days to Seedling Emergence (DSE), Dry Shoot Weight (DSW), Fresh Root Weight (FRW), Hypocotyle Diameter (HD), V4 Stage (V4)	*DSE.3.1, DSW.3.1, FRW.3.1, HD.3.1, V4.3.1*	LG10	Dry Shoot Weight(DSW), Fresh Root Weight (FRW), Fresh Shoot Weight (FSW)	*DSW.10.1, FRW.10.1, FSW.10.2*
LG3	Days to Seedling Emergence (DSE), Dry Shoot Weight (DSW), Fresh Root Weight (FRW), Fresh Shoot Weight (FSW), V4 Stage (V4)	*DSE.3.1, DSW.3.2, FRW.3.1, FSW.3.1, V4.3.1*	LG10	Dry Shoot Weight(DSW), Fresh Root Weight (FRW), Hypocotyle Diameter (HD), Shoot Length (Shoot L)	*DSW.10.1, FRW.10.1, FSW.10.2, HD.10.1, ShootL.10.1*
LG5	Days to seedling Emergence (DSE), V4 Stage (V4)	*DSE.5.1, V4.5.1*	LG12	Dry Root Weight (DRW), Shoot Length (Shoot L)	*DRW.12.1, ShootL.12.1*
LG5	Days to Seedling Emergence (DSE), Hypocotyle diameter (HD)	*DSE.5.2, HD.5.1, V4.5.2*	LG12	Dry Shoot Weight (DSW), Fresh Shoot Weight (FSW	*DSW.12.1, FSW.12.2*
			LG14	Dry Shoot Weight (DSW), V4 Stage (V4),	*DSW.14.1, V4.14.1*
LG7	hypocotyle Diameter (HD)	*HD.7.1, V4.7.1*	LG16	Hypocotyle Diameter (HD), Root Length (Root L)	*HD.16.1, RootL.16.1*
LG8	Dry Root Weigh (DRW), Root Length (Root L),	*DRW.8.1, RootL.8.1*	LG16	Dry Shoot Weight (DSW), Hypocotyle Diameter (HD)	*DSW.16.1, HD.16.2*
			LG17	Dry Shoot Weight (DSW), Fresh Root Weight (FRW), Dry Shoot Weight (DSW), Fresh Shoot Weigh (FSW)	*DSW.17.1, FRW.17.1, FSW.17.1*

for days to flowering (DSF) on linkage group 5 (nearest SSR marker; SSL231) was identified. This marker was also linked to the QTLs for several plant growth traits, and therefore could be beneficial for introgressing higher overall growth rate QTLs in the same genetic background.

To date, limited progress has been made in increasing germination and seedling vigour in sunflower. In this study we detected QTLs associated with these traits. Many QTLs associated with early growth and development were identified, although some QTLs were specific to one traits, several interesting regions were identified containing QTLs from more than one traits and overlapping with regions previously detected for growth and yield-related traits. Linkage group 10 contains several QTLs involved in all of the germination and seedling growth-related traits. The number of markers (SSRs or/and AFLPs) has to be increased especially in this linkage group at the interesting QTL interval. When these regions are more precisely mapped, the information obtained could help sunflower breeders in marker-assisted breeding programs. Knowledge of the accurate location of QTLs associated with seed germination and seedling vigour could offer opportunities for improvement of the efficiency of plant breeding and lines selection with improved seed and seedling vigour.

Materials and methods

Plant material and experimental design

116 F9 RILs derived from a cross between two sunflower inbred lines (PAC2 and RHA266) was used in present study. Developed RILs population through single seed descent (SSD) was kindly provided by INRA, France. RHA266 as paternal line was obtained from a cross between wild *H. annuus* and *peredovik* by USDA and PAC2 as maternal line is an INRA-France inbreed line selected from a cross between *H. petiolaris* and HA61 (Gentzbittel et al., 1995). This public RILs population has been widely used for genetic analysis of complex traits in sunflower (Rachid Al-Chaarani et al., 2004, 2005; Abou Al Fadil et al., 2007; Darvishzadeh et al., 2007; Poormohammad Kiani et al., 2007; 2009). RILs and their two parents were grown in a growth chamber under 25 ± 1ºC temperature and a day length of 16 h. Light intensity was $100\mu Em^{-2}s^{-1}$. The experiment was conducted in completely randomized design with three replications. Each replication comprised a 95 mm diameter Petri dish containing 20 seeds. Seeds were disinfected in a 5% calcium hypochloride solution and washed three times in distilled water. The seeds were then transferred to Petri dishes containing a triple layer of Whatman paper moistened with distilled water.

Number of germinated seed were determined daily until the time that the percentage of germination was stable in the two successive observations. Seeds were considered to have germinated when radicles were about 1 mm long (Khan et al., 2004). Speed of germination (Sger) as a good indicator of seed vigour was calculated according to the formula (Maguire, 1962):

$$Sger = \frac{\sum Gt}{\sum Dt}$$

where Gt= total germinated seeds; and Dt = total days.

Germinated seeds were transferred to special culture dishes (60×40×8cm) with double layer of filter papers moistened with distilled water. Thirteen days after transferring, the following parameters were assessed on sunflower seedling: shoot length (ShootL), root length (RootL), fresh root weight (FRW), fresh shoot weight (FSW), dry root weight (DRW) and dry shoot weight (DSW).

In another experiment the seedling growth and development was investigated using completely randomized design with three replications. Seeds of RILs and their two parents were surface-disinfected with 5% sodium hypochlorite solution for 15 minutes and rinsed three times with distilled water and planted in plastic pots in the same profound. Emergence as the appearance of the seedling shoot above the soil surface was determined based on the number of days; seedlings need to emerge. After seedling emergence, hypocotyle diameter (cm) was determined. Growth rates per plant were determined based on date that plant reached to growth stage V4 (four true leaves).

Data analysis and QTL detection

Analysis of variance (ANOVA) on data was performed using the general linear model (GLM) procedure of the SAS software version 9.2 (SAS Institute Inc.). The function "FREQ" of SPSS software (SPSS/PC-15, SPSS Inc.) was used to analyses the frequency distribution of population for the studied traits. The mean of RILs and their two parents was compared. Genetic gain when the mean of the 10% most relevant RILs was compared with the mean of their parents was determined. Genotypic and environmental variances as well as broad-sense heritability were calculated according to Kearsey and Pooni (1996) using least-square estimates of genetic parameters. The correlations among the different traits were determined.

The linkage map used in this study is the high-density SSR/AFLP genetic linkage map described recently by Poormohammad Kiani et al. (2007). The linkage map was constructed with 495 markers with the mean density of 3.7 cM per locus. QTLs were detected using the composite interval mapping (CIM) procedure by QTL Cartographer software, version 1.16 with model 6 of Zmapqtl (Basten et al., 2002). This model integrates two parameters for CIM: the number of markers which control the genetic background (*nm*=15) and a window size (*w*=15) that will block out a region of the genome on either side of the markers flanking the test site. The inclusion of background markers makes the analysis more sensitive to the presence of a QTL in the target interval. Additive effects of the detected QTLs and the percentage of phenotypic variation explained by each QTL (R^2) were estimated using the Zmapqtl program of QTL Cartographer (Basten et al., 2002).

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